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Effect of ultrasound irradiation on some Biophysical Parameters of Human Blood (*in vitro*)

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Abstract

Ultrasound is commonly employed in clinical practice as a part of physical therapy. In this study, an attempt has been made to investigate the effect of ultrasound irradiation on some Biophysical Parameters (pH, Conductivity, and Refractive index) of human blood. Blood samples of volume 5 ml were collected from the donors each time an experiment was performed. Blood was then transferred to heparin anticoagulant tubes. The human blood samples were divided into two parts, that is, control sample and sample for irradiation. The human blood sample was irradiated using an ultrasonic interferometer of 3MHz frequency for a duration of 1 h in an identical and controlled environment. The biophysical parameter was determined and compared with that of the control sample. Variations were observed in the values of pH, conductivity, and refractive index of ultrasonically irradiated human blood sample compared with that of the control sample. This study gives us an idea regarding the effect of ultrasound on some of the most important biophysical parameters of human blood. Further, the results obtained in the present study can be utilized in the future for the work on the biological effects of ultrasound and interpret the *in vivo* effects.

Keywords: Conductivity, Human blood, pH, Refractive index, Ultrasound

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Introduction

Ultrasonic biophysics is the study of the process that governs how ultrasound interacts with biological materials. The study of the bio-effects of ultrasound on biological materials can lead to therapeutic applications and risk evaluations for diagnostic ultrasound applications.[1] For many years, therapeutic applications of ultrasonography in medicine have been acknowledged and beneficial.[2] Ultrasound is frequently utilized as a therapeutic tool in lithotripsy of kidney stones, physiotherapy, tissue destruction in case of tumor treatment, and as a surgical tool in the form of high intensity focused ultrasound. It is also been used as a drug delivery system.[3] Current research shows that biomedical ultrasonography has a lot of potential for novel application and advancements in medicine. To make progress in the therapeutic uses of ultrasound, a deeper knowledge of the processes of tissue contact is required. The first biological effect of ultrasound was reported by Langevin in 1917. Ultrasonic absorption was seen in biological fluids such as water, milk at 1–10 GHz using the phenomenon of damped oscillation.[4] The influence of ultrasound on the living system was of thermal and mechanical destruction. The erythrocytes in the isotonic suspension were destroyed when treated with ultrasonic radiation.[5] Hemorrhage in mouse lung tissue after ultrasound exposure was observed since then, ultrasound-induced lung hemorrhage has been reported *in vivo* in mice,[6,7] rats.[8,9] Measuring the cell deformability in the cell at various pH ranging from 6.2 to 8.0 were studied and it was observed that there was a significant decrease in deformability of cells with lower pH. The observed red blood cell (RBC) deformability could be the consequence of pH affecting the cell membrane property.[10] The effect of ultrasound in cell suspensions is different from that in monolayers of cells attached to a surface and the probability of cavitation occurring in intact tissue depends on temperature, the tissue, and gas content.[11] Adverse health effects derived from cellular studies, animals, and clinical reports were reviewed to provide an insight into the *in vitro* and *in vivo* biological effects of ultrasound.[12] A review of the literature reveals that many studies have been done on the biological effects of ultrasound on animals, and cell suspensions, but the effect of ultrasound on pH, conductivity, and refractive index of human blood is scanty. Hence, it is in this perspective an effort has been made to study the effect of ultrasound irradiation on some biophysical parameters of human blood *in vitro*.

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Materials and Methods

Human Use and Regulatory status

The blood samples were taken from normal healthy human subjects with their consent every time an experiment was performed. This study was performed following the procedures formulated by National Ethical Guidelines for Biomedical and Health Research by Indian Council of Medical Research, New Delhi, and certifies that the studies on human blood *in vitro* were carried out in accordance with the principles of the declaration as laid down in the 1964 Declaration of Helsinki.

Blood Sample Collection

A human blood sample of volume 5 ml was collected from healthy donors with their consent, each time an experiment was performed. The collected blood sample was then transferred to Heparin tubes to prevent it from coagulation. A total of 30 blood samples (10 for pH study, 10 for electrical conductivity study, and 10 for refractive index study) were collected in the present study.

Exposure of Ultrasound

A variable path ultrasonic interferometer (Figure 1: Mittal Enterprises, Model M-81) of frequency 3 MHz has been utilized for irradiation. The blood sample to be irradiated to ultrasonic standing waves is filled into the measuring cell. The high-frequency generator excites the quartz crystal present at the bottom of the measuring cell at its resonant frequency which generates ultrasonic waves of 3 MHz frequency in the human blood sample. The sample was irradiated for a duration of 1 h.

For measurement of pH, a microcontroller-based pH meter (Figure 2: EQUIP-TRONICS Model EQ-621) was used. It can read pH both in automatic temperature compensation and manual temperature compensation mode. All the controls are feather touch with a digital display. The digital pH meter (EQUIP-TRONICS Model EQ-621) has a precision of ± 0.01%. It also has an electronic buffer for fault findings.

Initially, the blood sample whose pH was to be determined was taken in a cleaned and dry container. The pH electrode, dipped in distilled water was clamped on the provided holding clamp. It was ensured that there were no bubbles in the inner tube of the electrode. The BNC plug of the pH electrode and Temperature control probe was connected to their respective sockets. Once the Equip-tronics pH meter EQ-621 was turned on, the ATC probe temperature will be displayed on the screen. Now the instrument is calibrated using the buffer solutions of pH 7, 4, and 9.2. As the instrument is now calibrated, it is ready for measuring the pH of the given sample. Now the pH electrode was dipped in the human blood sample completely and the readings were recorded. The same procedure was followed for measuring the pH of ultrasonically irradiated human blood.

For measuring the electrical conductivity of human blood, a microcontroller-based digital conductivity meter (Figure 3: EQUIP-TRONICS Model-EQ-664A) was used which directly reads conductance in MHOS. The electrical conductivity meter (EQUIP-TRONICS Model-EQ-664A) has a precision of ± 1% ± last two digits.

Initially, the blood sample whose electrical conductivity is to be determined was reserved in a cleaned and dry glass container. The conductivity cell, having a cell constant of 1.00, was cleaned and soaked in distilled water for a minimum of 2 h before use. The conductivity cell was clamped on the provided holding clamp. The BNC plug of the conductivity cell is connected to the respective socket. Once the digital conductivity meter was switched on it displace the conductance value, value of conductance was calibrated to 1 mM using the special screwdriver provided with the instrument. Once the instrument is calibrated, the switch provided to measure the electrical conductivity was thrown up to read the electrical conductivity of a given sample in mS or μS. The electrical conductivity of blood was recorded by completely dipping the conductivity cell in the given blood sample. The same procedure was followed for measuring the conductivity of ultrasonically irradiated human blood.

In the present investigation, the refractive index was determined using Abbe's refractometer (Figure 4), which gives the value of the refractive index at an instant. The detailed theoretical and experimental set up of this instrument is provided somewhere else.[13] Further, it requires only a few drops of liquid for determining the refractive index which is the need for our present investigation. The refractive index of plasma for ultrasonically irradiated human blood was determined.

The plasma of ultrasonically irradiated human blood was obtained by centrifuging the whole blood at a rate of 1500 RPM for 15 min. The sodium light source, placed in front of the abbe's refractometer was turned on. The double prism of the refractometer was then opened and cleaned. After closing the double prism of the refractometer, using a pipette the space between the two prisms was filled with the plasma of ultrasonically irradiated human blood. Then the refractometers knob was turned till we get a clear interface between illuminated and dark regions. Further, the micrometer screw provided was utilized to refine the scale until the clear interface appeared. The refractive index value of plasma was then directly noted from the instrument.

Results

Table 1 reveals the data on the pH of normal and ultrasonically irradiated human blood for 1 h of 10 samples. The pH of human blood before ultrasound ranges from 7.01 to 7.68 with a mean value of 7.42. An increase in the value of pH was observed when the human blood was irradiated with ultrasound. The mean value of pH after irradiating with ultrasound is 7.61. The mean percentage increase in the value of pH is 2.63.

Table 2 presents the data on the electrical conductivity of normal and ultrasonically irradiated human blood for a duration of 1 h of 10 samples. The electrical conductivity of normal blood samples in the present study ranges from 3.76 mS to 4.61 mS, having a mean value of 4.19 mS. The present data reveal that the electrical conductivity of ultrasonically irradiated blood has

Table 1: Data on pH of normal and ultrasound irradiated human blood

S. No.	*Before exposure*	*After exposure*	*%variation for 60 min*
1.	7.60	7.77	2.23
2.	7.43	7.74	4.17
3.	7.54	7.81	3.58
4.	7.52	7.61	1.19
5.	7.47	7.69	2.94
6.	7.02	7.12	1.42
7.	7.63	7.86	3.01
8.	7.01	7.17	2.28
9.	7.68	7.82	1.82
10.	7.32	7.59	3.68
Mean	7.42	7.61	2.63

Table 2: Data on electrical conductivity (mS) of normal and ultrasound irradiated human blood

S. No.	*Before exposure*	*After exposure*	*%variation for 60 min*
1.	4.10	3.83	−6.58
2.	4.12	3.64	−11.6
3.	4.21	4.01	−4.75
4.	4.11	3.51	−14.5
5.	3.99	3.79	−5.01
6.	4.01	3.78	−5.73
7.	4.61	4.31	−6.50
8.	4.54	4.27	−5.94
9.	3.76	3.58	−4.78
10.	4.47	4.13	−7.60
Mean	4.19	3.88	−7.29

decreased. The mean value of electrical conductivity of human blood after irradiating it with ultrasound is 3.88 mS. The percent variation is −7.29 mS compared to the electrical conductivity of normal human blood.

Table 3 presents the data on the refractive index of plasma of ultrasonically irradiated human blood for a duration of 1 h of 10 samples. The refractive index of ultrasonically irradiated human blood samples varies from 1.354 to 1.362, whereas the refractive index of plasma of normal blood is 1.351.[13] Hence, the values of the refractive index of ultrasonically irradiated blood are slightly higher when compared to that of normal blood.

Discussion and Conclusion

Blood is valuable to mankind because without it life cannot exist. It is the most important constituent of the circulating system which helps in the normal functioning and growth of different parts of the human body. So any changes in human blood will effect the entire human body.

Table 3: Data on refractive index of ultrasound irradiated plasma

S. No.	*Refractive index of ultrasound irradiated plasma*
1.	1.359
2.	1.360
3.	1.362
4.	1.359
5.	1.359
6.	1.361
7.	1.356
8.	1.354
9.	1.361
10.	1.357
Mean	1.358

Figure 1: Ultrasonic interferometer

Figure 2: Digital pH meter (EQUIP-TRONICS Model EQ-621)

The alkalinity and acidity of blood can be described as pH and it is directly related to the ratio of H^+ and OH^- ions. The changes in the acid-base state of human blood can severely affect the normal functioning of the human body. The rise or fall in the concentration of CO_2 results in a decrease or increase in pH of human blood respectively. This increase or decrease in the pH of blood may affect the size and shape of RBC, Hb concentration, proteins of the erythrocyte membrane, and this may result in an effect on oxygen-carrying ability.[10] The pH value of normal human blood ranges between 7.35 and 7.45. Due to some pathological reasons there will be an increase and decrease in the pH values which results in the condition known as Alkalosis (pH >7.45) and Acidosis (pH <7.35) respectively. In the present investigation, the pH of normal and ultrasonically irradiated human blood was determined for 1 h using a digital pH meter. It is found that the pH of ultrasonically irradiated human blood was higher than normal human blood. The calculated mean value of ultrasonically irradiated human blood is 7.61 which is greater than 7.45, this may lead to a pathological condition known as Alkalosis. A condition known as echinocytosis (abnormal cell membrane) may result due to this increase in the value of pH. Kuzman *et al*. in the year 2000 reported that changes in pH may affect the membrane elastic properties.[10] The change in pH may affect the

Figure 3: Digital conductivity meter (EQUIP-TRONICS Model EQ-664A)

Figure 4: Abbe's refractometer

Osmotic fragility of the erythrocyte due to changes in both the erythrocyte membrane and cell water content.

The first study for the existence of the biological cell membrane was based on the electrical studies on cell suspension. To determine the impedance characteristics of blood and the physiological effects of electric currents on blood, a lot of studies have been made. As we know that blood is a complex fluid tissue, which contains protein solution and suspended cells in large numbers. There is a membrane known as the bilipid membrane of erythrocyte that separates the cell interior and cell exterior. This membrane acts as an electric insulator due to the presence of lipids. The charged particles, uncharged particles, and the varied count of three cells in the blood imparts one of the most important and interesting physical property, that is, conductivity. The electrical conductivity of biological fluids is the total contribution of all the cells and ions present in them. The result of the present study reveals that the electrical conductivity of ultrasonically irradiated human blood decreases in comparison to normal human blood. The average conductivity for ten normal human blood samples is 4.19 mS, whereas the mean conductivity after the exposure of ultrasound for 1 h is 3.88 mS. Ultrasound irradiation might have caused the bilipid membrane of the erythrocyte to offer a significant barrier to current flow. This variation in electrical conductivity affects the dielectric nature of human blood. The decrease in conductivity of human blood reflects that cell membranes permeability has been damaged along with the loss of electrolytes, ions, and intracellular components.[14]

Changes in the human body can initially be detected in the complete blood picture and urine analysis. In the field of medical therapy, the optical characteristics of biological tissues are of the most importance. The refractive index is an important parameter in the study of the optical properties of biological tissues. The refractive index profile of blood and plasma serum is broadly employed in clinical applications. In this day and age, most of the pathologist is interested in studying the refractive index profile of urine and blood. In the present investigation, the refractive index of plasma of ultrasonically irradiated human blood for 1 h was determined. Li *et al*., in the year 2000, determined the values of the refractive index of human blood samples of different groups using the TIR analysis.[15] The refractive index of plasma of ultrasonically irradiated human blood varies from 1.357 to 1.362. Here, the refractive index value of plasma of ultrasonically irradiated blood was compared with that of the normal plasma value of human blood which is 1.351.[13] It is observed that for ultrasonically irradiated human blood, the value of the refractive index of plasma is greater than that of normal blood. The study of the refractive index may be used as an important tool in biophysical investigations, which could help to determine the changes in the physiological state of human blood.

The present paper provides the data on the effect of ultrasound on some of the most important biophysical parameters of human blood. The results obtained in the present study can be utilized in the future for the work on the biological effects of ultrasound and interpret the *in vivo* effects.

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